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A Test of the Function of Juvenile Color Patterns in the Pomacentrid Fish *Hypsypops rubicundus* (Teleostei: Pomacentridae)[1]

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ABSTRACT: Color patterns of juveniles of many fish species differ from those of adult conspecifics. The adaptiveness of such coloration has seldom been studied, despite longstanding interest in the subject. I tested the hypothesis that distinctive juvenile coloration masks species identity, thereby reducing aggression from adults and permitting young fish to occupy areas within adult territories. I measured the responses of adult, territorial garibaldi (*Hypsypops rubicundus* [Girard]) to naturally colored juveniles and to juveniles altered to resemble adults or heterospecifics when presented live in clear, plastic bags filled with seawater. Preliminary results showed that adults attacked normally colored juveniles more than any other color pattern presented, indicating that juvenile coloration in *H. rubicundus* does not inherently reduce adult aggression. I discuss the merits and testability of alternative hypotheses for ontogenetic color change in damselfishes.

JUVENILE COLOR PATTERNS of many fish species differ from those of adults. Some juveniles and adults have even been misclassified as different species for many years (Rietschel 1975). Dramatic changes in color from juvenile to adult, termed ontogenetic color change (OCC), are exhibited by many animals other than fishes (Booth 1990). Various explanations have been proposed to account for OCC in fishes. When juveniles are dull and adults are bright, crypsis is commonly proposed as the reason for the juvenile coloration (deSylva 1963). However, the same hypothesis cannot apply when juveniles are colored more brightly than the adults, a pattern observed in many species of territorial reef fishes (Thresher 1984, Booth 1990).

Among territorial fishes, suitable habitats are commonly already occupied by adults (Sale 1980, Doherty 1983, Jones 1987). Moreover, because almost all reef fishes have planktonic larvae, there is very little chance of new recruits being related to nearby adults. Therefore, selection should favor traits in juveniles that allow them to coexist with adults. Recent investigators (Fricke 1980, Thresher 1984) have proposed that distinctive coloration in juvenile fishes may reduce attacks by territorial adult conspecifics, thereby enabling juveniles to gain access to resources found within adults' territories.

Fricke (1980) speculated that the distinctive coloration of juveniles of *Pomacanthus imperator* (Bloch) provides a kind of "intraspecific camouflage" that allows the juveniles to go unrecognized as competitors by the adults. Fricke simultaneously presented two juvenile-sized dummy fish, one painted like an adult and one like a juvenile, to both adults and juveniles. He found that adults responded mostly to the dummy with adult colors, while juveniles responded mostly to the dummy with juvenile colors.

Fricke's pioneering work suggests that adults respond differentially to differently colored models, but is "intraspecific camouflage" the explanation? There is considerable evi-

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dence to suggest that territorial damselfishes do use color pattern as a cue in species recognition, and that heterospecifics are attacked less than conspecifics (Brockman 1973, Myrberg and Thresher 1974, Kohda 1981, Harrington and Losey 1990). If juvenile coloration masks species identity from adults, adults should respond with reduced aggression to any color pattern that is distinctly different from the adult's own coloration. No other coloration of models was tested in Fricke's (1980) study.

To further test the idea that juvenile-specific coloration masks species identity, I experimented on a territorial damselfish that shows OCC, the garibaldi (*Hypsypops rubicundus* [Girard]). Garibaldi are commonly found between 2 and 20 m depth throughout Southern and Baja California in areas of high rocky relief (Limbaugh 1962). Adults of both sexes defend territories year-round, including shelter holes, grazing areas, and nest sites, for some males, from May through August. Garibaldi can grow to over 30 cm total length (TL) and live more than 13 yr (Clarke 1970). Mature individuals of both sexes are uniformly orange, and younger individuals (up to ca. 18 cm TL) bear many iridescent blue spots on an orange background. Adults and juveniles often co-exist within territories (personal observation), although not in all areas (Clarke 1970). I have seen predation on juveniles by large kelp bass, and adults are rarely taken by harbor seals and sea lions (Kritzler et al. 1950).

I tested whether the blue spots found on juvenile garibaldi mask species identity by simultaneously presenting to territorial adults the normally colored juveniles (blue spots), adult-colored juveniles (orange), and juveniles that were extensively altered in color to look like a different species. The responses given to each color of fish in this design were compared with those given to a slightly different combination that substituted black surfperch, *Embiotoca jacksoni* (Girard), for the extensively modified juvenile. If the normal juvenile coloration masks species identity, adults should respond with reduced aggression to the normal juvenile coloration, the extensively modified juvenile, and *E. jacksoni*.

MATERIALS AND METHODS

All experiments were conducted at Big Fisherman Cove, Catalina Island, California, from 31 June 1987 to 3 August 1987. Observations and tests were at a depth of 2 to 20 m using scuba gear. A distance of at least 3 m was maintained from all subjects while observing them, with brief interruptions to allow manipulation of treatment fish. Originally, I intended to use models, produced using the methods described in Helfman (1983). However, in preliminary tests models failed to elicit a measurable response from adult garibaldi. It seemed that movement was critical in triggering adult aggression, so I decided to use live fish. Treatment fish were captured at Cherry Cove, ca. 3 km from the selected test site, and were kept in aquaria until needed.

Territorial males were observed to locate nest sites and to compare topographies of different territories. Only adult males were used for testing because females were less distinctly territorial. I chose male territories within the test area that were similar in topography, and each male was in a similar stage of nesting (about 1 week after all eggs within the nest hatched and the larvae departed). Furthermore, at least one juvenile was present within each adult territory chosen for testing so that all adults had some prior experience with juveniles.

To ascertain the best distance for presenting differently colored treatment fish to adults, I first estimated the maximum distance to attack for five male territories (Myrberg and Thresher 1974). I placed a small adult male (20 cm TL) in a large, clear, weighted plastic bag filled with seawater. Starting at a distance of 5 m from the male's nest, the bag was moved in 1-m increments along a straight line into the territory. At each increment, the bag was allowed to remain for 5 min, and the behavior of the resident male was recorded. I used a fishless, water-filled bag as a control. The maximum distance to attack was estimated by observing where the bags were first attacked. Adult males attacked when the approaching fish was within 2 to 3 m of the center of their territories. Based on this estimate, I chose a distance of 1.5 m at which to

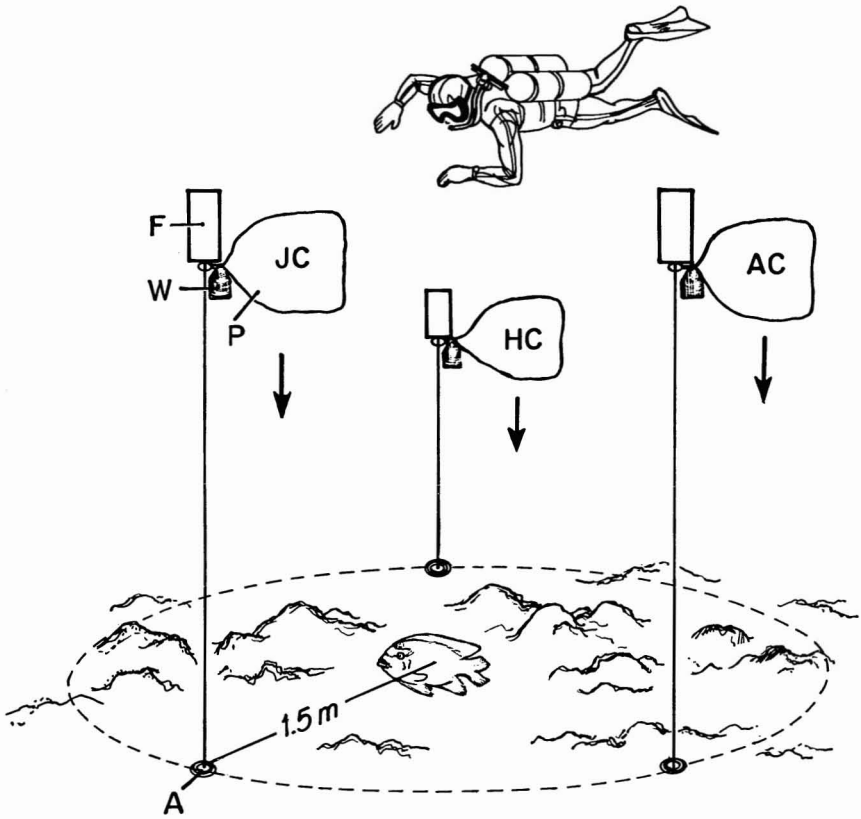

FIGURE 1. An example showing how differently colored treatment fish were presented to adult subjects. JC, normal-colored juvenile; HC, heterospecific-colored juvenile; AC, adult-colored juvenile (see text for further descriptions). Observations were collected from a position above the floats (diver). The position of the adult in the diagram indicates the location of the nest site. All plastic bags (P) containing fish were simultaneously released by two divers, allowing the weights on the bags (W) to sink the fish into the territory of the adult. Bags were anchored by means of a weight at the bottom (A) and a float at the top (F). The line distance between the float and the anchor varied, depending on how deep the subject fish was.

present the different treatment fish (described below). Thus, they were within attack distance but still far enough away that a clear choice could be made between individual treatment fish by the resident adult being tested.

Three color variations of equal-sized (ca. 16 cm TL) live fish were presented in clear, weighted, 25-liter plastic bags within the territory of an adult male at 1.5 m from the center of the territory (nest site). The bags were sealed to control for any olfactory cues that might affect the visual responses of the adult fish. Treatment fish were presented simultaneously over a period of 15 min by letting the bags drop from floats to the bottom (Figure 1). Every 5 min, I lifted treatment fish by pulling the bags up to the float (see Figure 1), rotated them to control for any positional effects within the territory, and then dropped them back into the territory. During the presentations, I recorded the approaches and attacks of the resident adult for each of the color variations. I introduced fresh seawater into the bags before each replicate.

Experiment 1 utilized modified and unmodified juvenile garibaldi in the process of changing to the adult color. Transitional juveniles maintain most of the original juvenile

color including the blue spots. As this phase progresses, these spots are lost rapidly and are replaced by bright orange. The change occurs over a relatively narrow size range (15–18 cm TL), based on measurements taken on juveniles collected at Catalina. Thus, similar-sized juveniles could be found (less than 1.5 cm TL difference) that differed in color, one retaining almost all of the original juvenile color, the other possessing nearly full adult color.

Color patterns in the first experiment were as follows: (1) normal juvenile color—an early-color-change juvenile, unmodified; (2) adult color—a late-color-change juvenile modified to look like an adult by using a forceps to remove the few scales that were still blue; (3) heterospecific color—a late-color-change juvenile with all traces of blue removed, then extensively modified by injecting colored dye (alcian blue [Kelly 1967]) in scale pockets on the body region. This resulted in a dark-brown-bodied individual with orange head and fins.

I controlled for any effects of color-pattern modification by injecting saline underneath the scales of fish and by removing similar numbers of orange scales from fish that did not have blue scales removed. Because of a limited supply of appropriately sized treatment fish, the same three individual fish were used as treatments when testing the responses of five different adults. However, I observed the fish both in aquaria before use and while being tested in bags and found no apparent differences in behavior between them. Thus, I felt confident that I could essentially treat them as living models.

Experiment 2 resembled the first, except that I substituted a black surfperch (*Embiotoca jacksoni*) for the heterospecific-colored juvenile in the first experiment. *E. jacksoni* is a dull, copper-colored fish, similar in shape to garibaldi. It is common near garibaldi territories and has some dietary overlap with adult garibaldi (M. Singer, pers. comm.). A 16-cm fish was chosen to match in size the other two treatment fish (adult and normal-colored *H. rubicundus* juveniles). Ten resident adults were used as subjects for this experiment.

I used a nonparametric analysis of variance (ANOVA) (Kruskal-Wallis) to test for significant differences in the total number of approaches and attacks between the different colors of treatment fish. Moreover, all possible pairwise comparisons for approaches and attacks were made using the nonparametric multiple comparisons test described in Zar (1974) and hereafter abbreviated as NMC.

RESULTS

Observations

Adults responded strongly to all conspecific juveniles presented to them, approaching and attacking all colors of treatment fish over the entire 15-min period (Table 1). Adults typically approached a treatment fish from the center of the territory and either displayed toward or attempted to bite the intruder once or several times. Afterward, adults usually returned to the center of their territory before approaching the same or a different treatment fish. Adults varied greatly with respect to the number of attacks delivered to the different treatment fish, even though most territory and nesting characteristics were equivalent. Treatment fish within bags responded similarly to approaches and attacks by adults, usually moving quickly away from the adult to the other side of the bag and lowering their

TABLE 1

RESPONSES (MEAN ± SD) OF ADULTS TO TREATMENT FISH PRESENTED IN CLEAR PLASTIC BAGS

TREATMENT FISH	APPROACHES	ATTACKS
Experiment 1		
AC	10.2 ± 3.3	27.8 ± 22.7
HC	10.2 ± 1.9	27.8 ± 21.4
JC	14.4 ± 3.6	48.8 ± 23.5
Experiment 2		
AC	17.4 ± 4.7	60.7 ± 13.0
EJ	6.3 ± 4.4	8.8 ± 10.8
JC	16.6 ± 3.8	87.9 ± 32.6

NOTE: Five subjects in experiment 1, 10 in experiment 2. See results in text for significance tests. AC, adult coloration; HC, heterospecific coloration; JC, juvenile coloration; EJ, heterospecific (*Embiotoca jacksoni*).

median fins. Although it was not quantified, the treatment fish did not seem to change their behavior through the course of each experiment in which they were used.

Attack Behavior

In both experiments, adults exhibited significant differences in attack behavior to the differently colored test fish ($P < 0.05$ for all treatment fish in experiment 1, $\chi^2 = 6.23$, df = 2; $P < 0.001$ for experiment 2, $\chi^2 = 21.23$, df = 2; Kruskal-Wallis ANOVA). Moreover, adults attacked the juvenile color pattern (JC) significantly more than the other color patterns ($P < 0.05$ in both experiments 1 and 2; NMC test comparing JC against all possible pairs). In experiment 1 the attack rate on the heterospecific-colored juvenile (HC) was indistinguishable from that on the conspecific adult-colored juvenile (AC) (mean number of attacks for AC and HC = 27.8, $P = 0.8$; NMC). In contrast, the true heterospecific, *Embiotoca jacksoni* (EJ), elicited a very low response in experiment 2 (Table 1).

Approach Behavior

The number of approaches to the three color variations presented in experiment 1 did not differ significantly ($P > 0.07$; $\chi^2 = 5.07$; df = 2; Kruskal-Wallis). Adults, therefore, were detecting and sampling each of the color variations at similar rates. Experiment 2 showed a slightly different pattern. Although both of the *H. rubicundus* juveniles were approached equally, the number of approaches to *E. jacksoni* was significantly lower ($P = 0.06$ for AC against JC, $P < 0.01$ for EJ against AC and JC; NMC). The response to *E. jacksoni* was unusual in that some adults responded aggressively but others ignored it entirely.

DISCUSSION

If natural juvenile coloration of *H. rubicundus* masks species identity, adults should have attacked the normal-colored juvenile, the extensively modified juvenile, and *E. jacksoni* less than the adult-colored juvenile. However, results indicated otherwise. Adults attacked the normal-colored juvenile most frequently. Moreover, adults responded the same to heterospecific-colored juveniles as to adult-colored juveniles, and the true heterospecific, *E. jacksoni*, was only occasionally attacked. The lower response toward *E. jacksoni*, a true heterospecific, is consistent with data gathered on other pomacentrids (Brockmann 1973, Myrberg and Thresher 1974, Kohda 1981, Harrington and Losey 1990). I attempted to follow Fricke's (1980) methods in using only one of each type of "model" to test the species camouflage hypothesis, but an argument can be made that because individual models (test fish) were not replicated, the study only reveals the behavior of adults to one test fish and not to an entire population of test fishes (Hurlbert 1984). Thus, these findings should be considered preliminary in nature. However, the main variability between test fishes would most likely be behavioral (I have examined over 40 juveniles and found that their color patterns have little variability within a stage), and although I do not deny the potential importance of possible behavioral differences between test fish, my focus in this study was purely on the effects of color on behavior.

Although intraspecific camouflage is not supported, another hypothesis termed "adult-habituation" (Thresher 1984) may explain the observation of high attack rates on juveniles in garibaldi. Moreover, it gives a theoretical basis for why juveniles might be more brightly colored than adults. Thresher proposed that when juveniles can remain within adult territories by using shelter holes too small for the adults to follow, the bright colors of the juveniles promote faster habituation of the adult to the presence of the juvenile. This allows the juvenile eventually to increase the amount of time it can spend foraging within the adult's territory. This might also explain the dominance of bright colors on juveniles among species where the juvenile is distinctly colored.

There is evidence to support the hypothesis that bright colors speed habituation (Gittleman et al. 1980). Bright colors often advertise

distastefulness (Cott 1957), and if gaudy colors are more quickly learned by predators as representing noxious prey, the same learning mechanism could apply to adults learning to avoid wasting time chasing juvenile fish. From an energetic standpoint, it would profit an adult to learn quickly that it cannot force a juvenile from its territory if it has access to small shelter holes. The use of shelter holes that allow access only to juveniles has been observed in a number of fishes (Sale 1980), and the same pattern is shown in *Hypsypops*. Thus, a color adopted by the juvenile that expedites this learning process may be profitable to both the adult and the juvenile. Alternatively, once the juvenile color pattern has been habituated to, any subsequent juveniles should be able to gain access to the territory readily, whether small shelter holes are available or not. If juveniles compete with adults for resources, then habituation would not be favored by selection because any adult who habituated to juvenile color patterns would be inundated by competitors. Cheaters would also prosper in this system, because any individuals that maintained the juvenile coloration into the adult stage would be less subject to aggression by neighbors already habituated to the juvenile color pattern. In this case, the system would be unstable.

Thresher (1984) cited as evidence supporting the habituation hypothesis his observation in *Eupomacentrus rectifraenum* (Gill) (now *Stegastes rectifraenum*) of an inverse relationship between the number of juveniles residing in a territory and the attack rate of a territory-holding adult against an unfamiliar juvenile presented in a glass bottle. Those results, however, do not provide direct evidence that it is the color of the juveniles that caused habituation or increased the rate. Adults may habituate to the presence of any fish that remains in the area long enough and does not seem to pose a substantial threat. Data on the habituation rates of adults to juveniles of different colors that can still use shelter holes may be the only way to test this hypothesis directly.

Regardless of the mechanism involved, any hypothesis that attempts to explain bright juvenile colors in terms of access to adult territories must first explain how such a system could have evolved if juveniles are a serious threat to territorial resources. Some evidence (Rasa 1968) suggests that juveniles of some territorial damselfishes do not compete for food or other resources with adults, but other evidence (Clarke 1970, Myrberg and Thresher 1974, Jones 1987) suggests that diets overlap in some. However, no data indicate the extent to which juveniles may diminish the resources within an adult's territory. The relatively large (compared with juveniles) adult size of most species of damselfish that show OCC suggests qualitative as well as quantitative differences in the utilization of resources (e.g., juveniles may use small shelter holes that are not utilizable by adults).

If juveniles are not a substantial threat to adult territories and adults occupy most of the habitats suitable for juveniles, then selection would favor mechanisms to recognize age or size classes. Juveniles would potentially benefit from decreased aggression, and adults from a decrease in the amount of time spent defending the territory against inconsequential competitors.

In addition to increased access to food (provided adults are monopolizing good feeding areas), residing within adult territories could confer other benefits. Adults defend their territories against fish many times their size (Clarke 1970), so juveniles may obtain some safety from predators by occupying areas near adults. Juveniles may also benefit from associating with a territorial adult by a decrease in the attacks given to them by neighboring conspecific adults. This was observed by Rohwer (1982) in Harris's sparrows, but has yet to be shown for any species of fish.

Few alternative hypotheses have been offered to explain OCC in damselfishes. Intra-juvenile advertisement has been proposed for fishes whose juveniles exhibit territoriality (Thresher 1984). Although juvenile garibaldi exhibit aggressive behavior toward one another in aquaria (unpublished data), this might be an artifact of the confines of the aquaria; it is not clear whether juveniles do so in the field. I have observed them sharing shelter holes on the reef, suggesting that they are not agonistic toward one another.

Alternatively, if juveniles can reduce adult attacks by increasing the number of juveniles within a territory (dilution effect), then juvenile advertisement may operate to attract additional juveniles to a territory, up to the point where overcrowding would become a problem (Stamps 1988). Data on the preference of juveniles for settling in territories with and without juveniles would resolve this hypothesis.

Unpublished data from preliminary aquarium studies of adult garibaldi suggest pronounced individual differences in aggressive responses toward conspecifics. Thus, the observation of juveniles within adult territories may simply reflect reduced aggression on the part of those adults, although only comparison with adults with no juveniles in their territories could test this idea.

Last, naming a color pattern cryptic or conspicuous is a function of human perception (Endler 1978). Coloration that appears conspicuous to humans may be cryptic to the primary predator of that species, or even to the species itself. It is possible that bright juvenile colorations in damselfishes may actually be cryptic. Many factors need to be measured, often difficult in the field, to adequately determine the conspicuousness of a color pattern.

The study reported here is only preliminary, but I hope that it will generate more interest in the subject. Although the focus was on the effects of color alone on behavior, it is likely that OCC in damselfishes is attributable to more than one factor. This study did not attempt to control for potential behavioral or other differences between individual "model" juvenile fishes used in the study, and it is unclear what role prior experience plays in the behavior adults exhibit toward juveniles. Moreover, many of the alternative hypotheses proposed here are not mutually exclusive, and future research should focus on an approach utilizing multivariate techniques.

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